Binding Affinity of GM3 Lactone to Wheat Germ Agglutinin

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Binding amounts and initial binding rates of Wheat Germ Agglutinin (WGA) to GM3 lactone containing mixed monolayer were measured by using a quartz-crystal microbalance (QCM). GM3 lactone showed the higher initial binding rate and binding amount compared with the parent GM3.

Gangliosides are sialic acid-containing glycolipids that are widely present in cell membranes. GM3 has a sialosyl residue attached to a galactose group of lactosyl moiety linked to a ceramide (Figure 1A). GM3 is known as an antigen on melanoma cells,[1] a receptor for influenza virus,[2] and a cell adhesion molecule.[3] When subjected to mild acidic condition, gangliosides will form internal esters, so called "lactone", between carboxyl group of sialic acid and hydroxyl group of a neighboring saccharide.[4] Ganglioside lactones have been detected in brains of mouse,[5] rat,[5] human,[6] and whale.[7] An IgM monoclonal antibody established after immunization with B16 melanoma showed stronger affinity with GM3 lactone than with GM3.[8] Therefore, it is considered that the real immunogen in B16 melanoma could be GM3 lactone. The recognition functions of GM3 lactone, however, has not been investigated so much.

GM3 and GlcCer were obtained from Snow Brand Milk Products Co., Ltd., Japan, and sphingomyelin (SM) was purchased from Sigma Co., Ltd., USA. WGA (Mw = 43200), which can bind saccharides such as sialic acid and *N*-acetylglucosamine (GlcNAc) etc., was purchased from Seikagaku Co., Japan. Those were used without further purification. GM3 lactone was prepared from GM3 in glacial acetic acid according to the method of Yu et al.[9] Formation of GM3 lactone was observed with CD spectrometry.[10] The purity was confirmed by TLC (chloroform : methanol : 0.2% calcium chloride = 6 : 4 : 1 v/v, or n-propanol : ethyl acetate : chloroform : methanol : water = 25 : 25 : 25 : 10 : 9 v/v). The degree of the lactonization was 80-90%. Major component of lactone bond was NeuAc1 → 2 in 90%, and the rest was NeuAc1 → 4.[7] The chemical structure of major GM3 lactone was shown in Figure 1A. The GM3 lactone was solubilized in mixed organic solvent (chloroform : methanol = 4 : 1). The solution was stored at -2 °C. When the GM3 lactone was dispersed in phosphate (pH 7.2) at 20 °C, the ratio of GM3 lactone and GM3 was not changed at least for 8 h.

Preparation of a GM3 lactone-containing monolayer and quantitative analysis of WGA-binding to the monolayer was done according to the previous paper.[11,12] A mixed solvent of chloroform and methanol containing glycolipid or SM, was spread on aqueous solution (10 mM phosphate buffer, pH 7.2) in a Teflon-coated trough with a microcomputer-controlled Teflon barrier (USI, Fukuoka, Japan). A QCM plate was attached horizontally on the mixed monolayer at the surface pressure of 30 mN m^{-1}. The frequency decrease of the QCM (mass increase) responding to the addition of WGA in the aqueous solution was

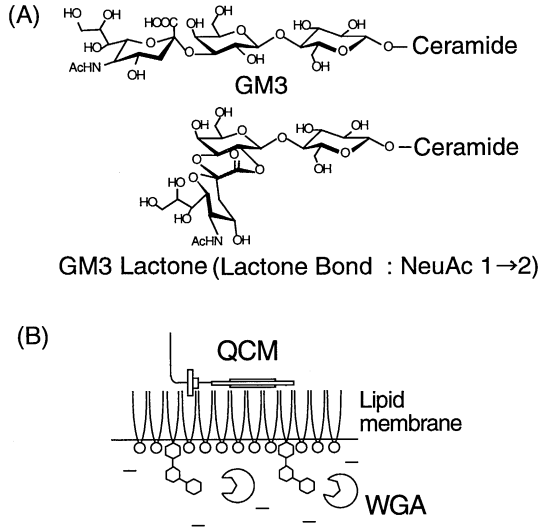

Figure 1. (A) Chemical structures of ganglioside (GM3) and GM3 lactone and (B) an experimantal apparatus of a QCM attached horizontally on a monolayer.

followed with time. A notional illustration for a experimental apparatus is shown in Figure 1B. The QCM employed is 9 MHz AT-cut quartz. Calibration showed that a frequency decrease of 1 Hz corresponded to a mass increase of 0.5 ng on the QCM electrode at an air-water interface.

Figure 2 shows typical time courses of frequency changes of the QCM for 10 mol% of GM3 and GM3 lactone reconstituted in SM matrix responding to the addition of WGA (5 ppm) into the subphase. The frequency decreased with time by the addition of WGA and saturated within 80 min. Binding amount of WGA to GM3 lactone was significantly higher than that to the parent GM3 in the SM matrix. Lactonization of GM3 resulted in the

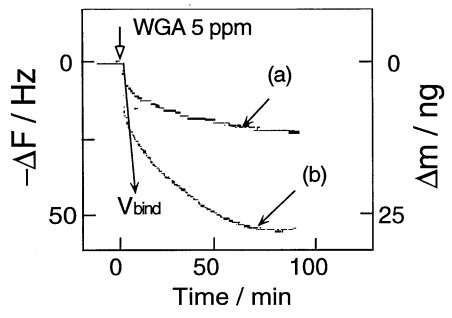

Figure 2. Time-courses of frequency decrease (-ΔF) and mass increase (Δm) of the QCM attached on (a) GM3/SM (10 : 90 by mol%) and (b) GM3 lactone/SM (10 : 90 by mol%) monolayers.

increased binding affinity for WGA. The presence of 10 mM GlcNAc in the aqueous phase inhibited the binding of WGA to the GM3 lactone-containing membrane.

When the concentration of WGA in the subphase was increased, the binding amount showed simple saturation curves. Maximum binding amounts (Δm_{max}) and binding constants (K_a) were obtained from reciprocal plots according to the method described in the previous paper.[11] The K_a values for GM3 and GM3 lactone were order of 10^7 M^{-1}. No significant defferences between GM3 and GM3 lactone were observed. Figure 3A shows the Δm_{max} of WGA bound to GM3 lactones which were reconstituted in GlcCer and SM matrices. The Δm_{max} of WGA to GM3 lactone in two matrices were resemble. We have reported that the Δm_{max} for GM3/SM monolayers were significantly lower than those for GM3/GlcCer monolayers (Figure 3B).[11] The GM3-SM interaction was considered to alter the oligosaccharide orientation, and make the recognition of GM3 hidden.[11,12] The GM3-SM interaction may be caused by complemental packing due to molecular shape and ionic interaction. Lactonization of GM3 will alter the oligosaccharide conformation and disappear the anionic charge. Therefore, Figure 3 suggested that lactonization of GM3 decreased the interaction between GM3 and SM.

The initial binding rates (V_{bind}) for the GM3 lactone/GlcCer monolayer were significantly higher than those for the GM3 lactone/SM monolayer (Figure 4A). The V_{bind} for the GM3 lactone/GlcCer monolayer was also significantly higher compared with those for the parent GM3/GlcCer monolayer previously reported (Figure 4B).[11]

It has been reported that inner esterification of ganglioside such as GM3[9] and GD3[14] causes conformational changes and structural rigidity. Furthermore, Maggio et al demonstrated that intermolecular packing and surface potential of GD3 lactone in the monolayer were different from those of the parent GD3.[15] WGA is known to bind with *N*-acetyl group of sialic acid.

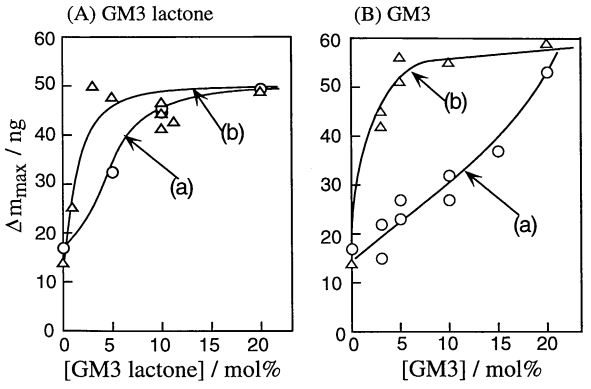

Figure 3. The maximum binding amount (Δm_{max}) of WGA to GM3 lactone (A) and GM3 (B) reconstituted in SM (a) and GlcCer (b) matrix lipids as a function of mole percentages of gangliosides.

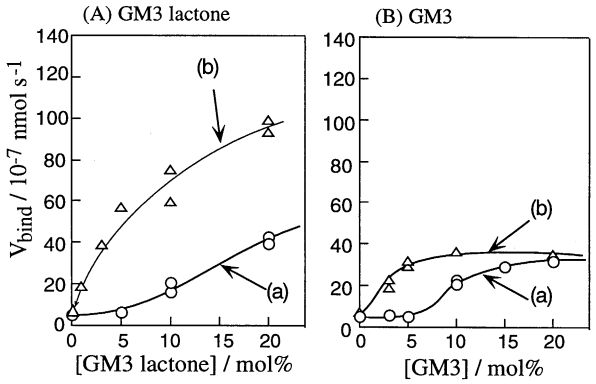

Figure 4. The binding rate (V_{bind}) of WGA to GM3 lactone (A) and GM3 (B) reconstituted in SM (a) and GlcCer (b) matrix lipids as a function of mole percentages of gangliosides.

Binding behavior of WGA to ganglioside membranes must be reflected by the direction of *N*-acetyl group of sialic acid exposed to the water phase. Lactonization of sialyllactose could alter the direction of *N*-acetyl group of sialic acid. We can expect that conformational changes of oligosaccharide by lactonization will lead to the modulation of recognition function of gangliosides on monolayers.

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